Figure 1

```
ATATTGCTGAGCTCAGGGAGTGAGGGCCCCACATTTGAGACAGTGAGCCCCAAGAAGAGG   60

GATCCCTGCTCCAGCAGCTGCAAGGTGCAAGAAGAAGAAGATCCCAGGGAGGAAAATGTG   120
                                                         M  C    2

CTGGAGACCCCTGTGTCGGTTCCTGTGGCTTTGGTCCTATCTGTCTTATGTTCAAGCAGT   180
 M  R  P  L  C  R  F  L  W  L  W  S  Y  L  S  Y  V  Q  A  V     22

GCCTATCCAGAAAGTCCAGGATGACACCAAAACCCTCATCAAGACCATTGTCACCAGGAT   240
 P  I  Q  K  V  Q  D  D  T  K  T  L  I  K  T  I  V  T  R  I     42

CAATGACATTTCACACACGCAGTCGGTATCCGCCAAGCAGAGGGTCACTGGCTTGGACTT   300
 N  D  I  S  H  T  Q  S  V  S  A  K  Q  R  V  T  G  L  D  F     62

CATTCCTGGGCTTCACCCCATTCTGAGTTTGTCCAAGATGGACCAGACTCTGGCAGTCTA   360
 I  P  G  L  H  P  I  L  S  L  S  K  M  D  Q  T  L  A  V  Y     82

TCAACAGGTCCTCACCAGCCTGCCTTCCCAAAATGTGCTGCAGATAGCCAATGACCTGGA   420
 Q  Q  V  L  T  S  L  P  S  Q  N  V  L  Q  I  A  N  D  L  E    102

GAATCTCCGAGACCTCCTCCATCTGCTGGCCTTCTCCAAGAGCTGCTCCCTGCCTCAGAC   480
 N  L  R  D  L  L  H  L  L  A  F  S  K  S  C  S  L  P  Q  T    122

CAGTGGCCTGCAGAAGCCAGAGAGCCTGGATGGCGTCCTGGAAGCCTCACTCTACTCCAC   540
 S  G  L  Q  K  P  E  S  L  D  G  V  L  E  A  S  L  Y  S  T    142

AGAGGTGGTGGCTTTGAGCAGGCTGCAGGGCTCTCTGCAGGACATTCTTCAACAGTTGGA   600
 E  V  V  A  L  S  R  L  Q  G  S  L  Q  D  I  L  Q  Q  L  D    162

TGTTAGCCCTGAATGCTGAAGTTTCAAAGGCCACCAGGCTCCCAAGAATCATGTAGAGGG   660
 V  S  P  E  C  *                                               167

AAGAAACCTTGGCTTCCAGGGGTCTTCAGGAGAAGAGAGCCATGTGCACACATCCATCAT   720
TCATTTCTCTCCCTCCTGTAGACCACCCATCCAAAGGCATGACTCCACAATGCTTGACTC   780
AAGTTATCCACACAACTTCATGAGCACAAGGAGGGGCCAGCCTGCAGAGGGGACTCTCAC   840
CTAGTTCTTCAGCAAGTAGAGATAAGAGCCATCCCATCCCCTCCATGTCCCACCTGCTCC   900
GGGTACATGTTCCTCCGTGGGTACACGCTTCGCTGCGGCCCAGGAGAGGTGAGGTAGGGA   960
TGGGTAGAGCCTTTGGGCTGTCTCAGAGTCTTTGGGAGCACCGTGAAGGCTGCATCCACA   1020
CACAGCTGGAAACTCCCAAGCAGCACACGATGGAAGCACTTATTTATTTATTCTGCATTC   1080
TATTTTGGATGGATCTGAAGCAAGGCATCAGCTTTTTCAGGCTTTGGGGGTCAGCCAGGA   1140
TGAGGAAGGCTCCTGGGGTGCTGCTTTCAATCCTATTGATGGGTCTGCCCGAGGCAAACC   1200
TAATTTTTGAGTGACTGGAAGGAAGGTTGGGATCTTCCAAACAAGAGTCTATGCAGGTAG   1260
CGCTCAAGATTGACCTCTGGTGACTGGTTTTGTTTCTATTGTGACTGACTCTATCCAAAC   1320
ACGTTTGCAGCGGCATTGCCGGGAGCATAGGCTAGGTTATTATCAAAAGCAGATGAATTT   1380
TGTCAAGTGTAATATGTATCTATGTGCACCTGAGGGTAGAGGATGTGTTAGAGGGAGGGT   1440
GAAGGATCCGGAAGTGTTCTCTGAATTACATATGTGTGGTAGGCTTTTCTGAAAGGGTGA   1500
GGCATTTTCTTACCTCTGTGGCCACATAGTGTGGCTTTGTGAAAAGGACAAAGGAGTTGA   1560
CTCTTTCCGGAACATTTGGAGTGTACCAGGCACCCTTGGAGGGGCTAAAGCTACAGGCCT   1620
TTTGTTGGCATATTGCTGAGCTCAGGGAGTGAGGGCCCCACATTTGAGACAGTGAGCCCC   1680
AAGAAAAGGGTCCCTGGTGTAGATCTCCAAGGTTGTCCAGGGTTGATCTCACAATGCGTT   1740
TCTTAAGCAGGTAGACGTTTGCATGCCAATATGTGGTTCTCATCTGATTGGTTCATCCAA   1800
AGTAGAACCCTGTCTCCCACCCATTCTGTGGGGAGTTTTGTTCCAGTGGGAATGAGAAAT   1860
CACTTAGCAGATGGTCCTGAGCCCTGGGCCAGCACTGCTGAGGAAGTGCCAGGGCCCCAG   1920
GCCAGGCTGCCAGAATTGCCCTTCGGGCTGGAGGATGAACAAAGGGGCTTGGGTTTTTCC   1980
ATCACCCCTGCACCCTATGTCACCATCAAACTGGGGGGCAGATCAGTGAGAGGACACTTG   2040
ATGGAAAGCAATACACTTTAAGACTGAGCACAGTTTCGTGCTCAGCTCTGTCTGGTGCTG   2100
TGAGCTAGAGAAGCTCACCACATACATATAAAAATCAGAGGCTCATGTCCCTGTGGTTAG   2160
ACCCTACTCGCGGCGGTGTACTCCACCACAGCAGCACCGCACCGCTGGAAGTACAGTGCT   2220
GTCTTCAACAGGTGTGAAAGAACCTGAGCTGAGGGTGACAGTGCCCAGGGGAACCCTGCT   2280
TGCAGTCTATTGCATTTACATACCGCATTTCAGGGCACATTAGCATCCACTCCTATGGTA   2340
GCACACTGTTGACAATAGGACAAGGGATAGGGGTTGACTATCCCTTATCCAAAATGCTTG   2400
GGACTAGAAGAGTTTTGGATTTTAGAGTCTTTTCAGGCATAGGTATATTTGAGTATATAT   2460
AAAATGAGATATCTTGGGGATGGGGCCCAAGTATAAACATGAAGTTCATTTATATTTCAT   2520
AATACCGTATAGACACTGCTTGAAGTGTAGTTTTATACAGTGTTTTAAATAACGTTGTAT   2580
GCATGAAAGACGTTTTTACAGCATGAACCTGTCTACTCATGCCAGCACTCAAAAACCTTG   2640
GGGTTTTGGAGCAGTTTGGATCTTGGCTTTTCTGTTAAGAGATGGTTAGCTTATACCTAA   2700
AACCATAATGGCAAACAGGCTGCAGGACCAGACTGGATCCTCAGCCCTGAAGTGTGCCCT   2760
TCCAGCCAGGTCATACCCTGTGGAGGTGAGCGGGATCAGGTTTTGTGGTGCTAAGAGAGG   2820
AGTTGGAGGTAGATTTTGGAGGATCTGAGGGC                               2852
```

Figure 2

```
---G--GTTG CAAGGCCCAA GAAGCCCA-- -TCCTGGGAA GGAAAATGCA      50
TTGGGGAACC CTGTG-CGGA TTCTTGTGGC TTTGGCCCTA TCTTTTCTAT     100
GTCCAAGCTG TGCCCATCCA AAAAGTCCAA GATGACACCA AAACCCTCAT     150
CAAGACAATT GTCACCAGGA TCAATGACAT TTCACACACG CAGTCAGTCT     200
CCTCCAAACA GAAAGTCACC GGTTTGGACT TCATTCCTGG GCTCCACCCC     250
ATCCTGACCT TATCCAAGAT GGACCAGACA CTGGCAGTCT ACCAACAGAT     300
CCTCACCAGT ATGCCTTCCA GAAACGTGAT CCAAATATCC AACGACCTGG     350
AGAACCTCCG GGATCTTCTT CACGTGCTGG CCTTCTCTAA GAGCTGCCAC     400
TTGCCCTGGG CCAGTGGCCT GGAGACCTTG GACAGCCTGG GGGGTGTCCT     450
GGAAGCTTCA GGCTACTCCA CAGAGGTGGT GGCCCTGAGC AGGCTGCAGG     500
GGTCTCTGCA GGACATGCTG TGGCAGCTGG ACCTCAGCCC TGGGTGCTGA     550
GGCCTTGAAG GTCACTCTTC CTGCAAGGAC T-ACGTTAAG GGAAGGAACT     600
CTGGTTTCCA GGTATCTCCA GGATTGAAGA GCATTGCATG GACACCCCTT     650
ATCCAGGACT CTGTCAATTT CCCTGACTCC TCTAAGCCAC TCTTCCAAAG     700
G                                                         701
```

Figure 3

1	Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr
16	Leu Phe Tyr Val Gln Ala↓Val Pro Ile Gln Lys Val Gln Asp Asp
31	Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
46	Ser His Thr Gln Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu
61	Asp Phe Ile Pro Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met
76	Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile Leu Thr Ser Met Pro
91	Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu Glu Asn Leu Arg
106	Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys His Leu Pro
121	Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly Val Leu
136	Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu
151	Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
166	Gly Cys End

Figure 4

```
Mouse   MCWRPLCRFL WLWSYLSYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS    50
        * **  *       *  *
Human   MHWGTLCGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS

Mouse   VSAKQRVTGL DFIPGLHPIL SLSKMDQTLA VYQQVLTSLP SQNVLQIAND   100
          *                   -           -   -     *     *
Human   VSSKQKVTGL DFIPGLHPIL TLSKMDQTLA VYQQILTSMP SRNVIQISND

Mouse   LENLRDLLHL LAFSKSCSLP QTSGLQKPES LDGVLEASLY STEVVALSRL   150
                -           *   **   ***-   *       *
Human   LENLRDLLHV LAFSKSCHLP WASGLETLDS LGGVLEASGY STEVVALSRL

Mouse   QGSLQDILQQ LDVSPEC                                      167
              - *    -  *
Human   QGSLQDMLWQ LDLSPGC
```

Figure 5

1	Met Cys Trp Arg Pro Leu Cys Arg Phe Leu Trp Leu Trp Ser Tyr
16	Leu Ser Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
31	Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
46	Ser His Thr Ser Val Ser Ala Lys Gln Arg Val Thr Gly Leu Asp
61	Phe Ile Pro Gly Leu His Pro Ile Leu Ser Leu Ser Lys Met Asp
76	Gln Thr Leu Ala Val Tyr Gln Gln Val Leu Thr Ser Leu Pro Ser
91	Gln Asn Val Leu Gln Ile Ala Asn Asp Leu Glu Asn Leu Arg Asp
106	Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys Ser Leu Pro Gln
121	Thr Ser Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly Val Leu Glu
136	Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu Gln
151	Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Val Ser Pro Glu
166	Cys End

Figure 6

```
  1  Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr
 16  Leu Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
 31  Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
 46  Ser His Thr Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu Asp
 61  Phe Ile Pro Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met Asp
 76  Gln Thr Leu Ala Val Tyr Gln Gln Ile Leu Thr Ser Met Pro Ser
 91  Arg Asn Val Ile Gln Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp
106  Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys His Leu Pro Trp
121  Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly Val Leu Glu
136  Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu Gln
151  Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro Gly
166  Cys End
```

Figure 7

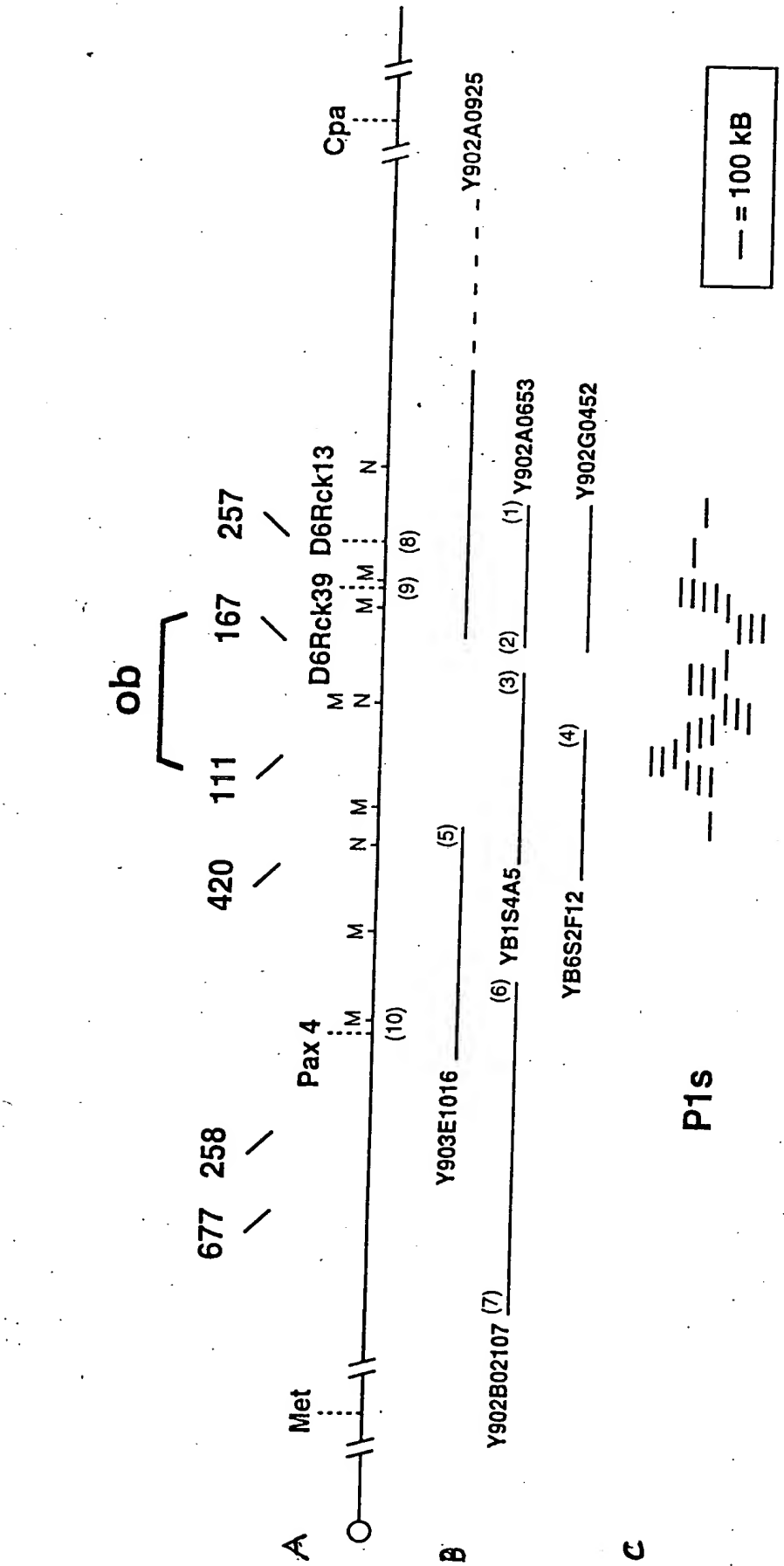

ob
677
258
420
111
167
257
Met
Pax 4
D6Rck39
D6Rck13
Cpa
M
N
M
N
M
N
M
M
M
N
(10)
(9)
(8)
A
Y903E1016
(5)
Y902B02107
(7)
(6)
YB1S4A5
(3)
YB6S2F12
(4)
Y902A0925
(1)
Y902A0653
(2)
Y902G0452
B
P1s
C
= 100 kB

Figure 8

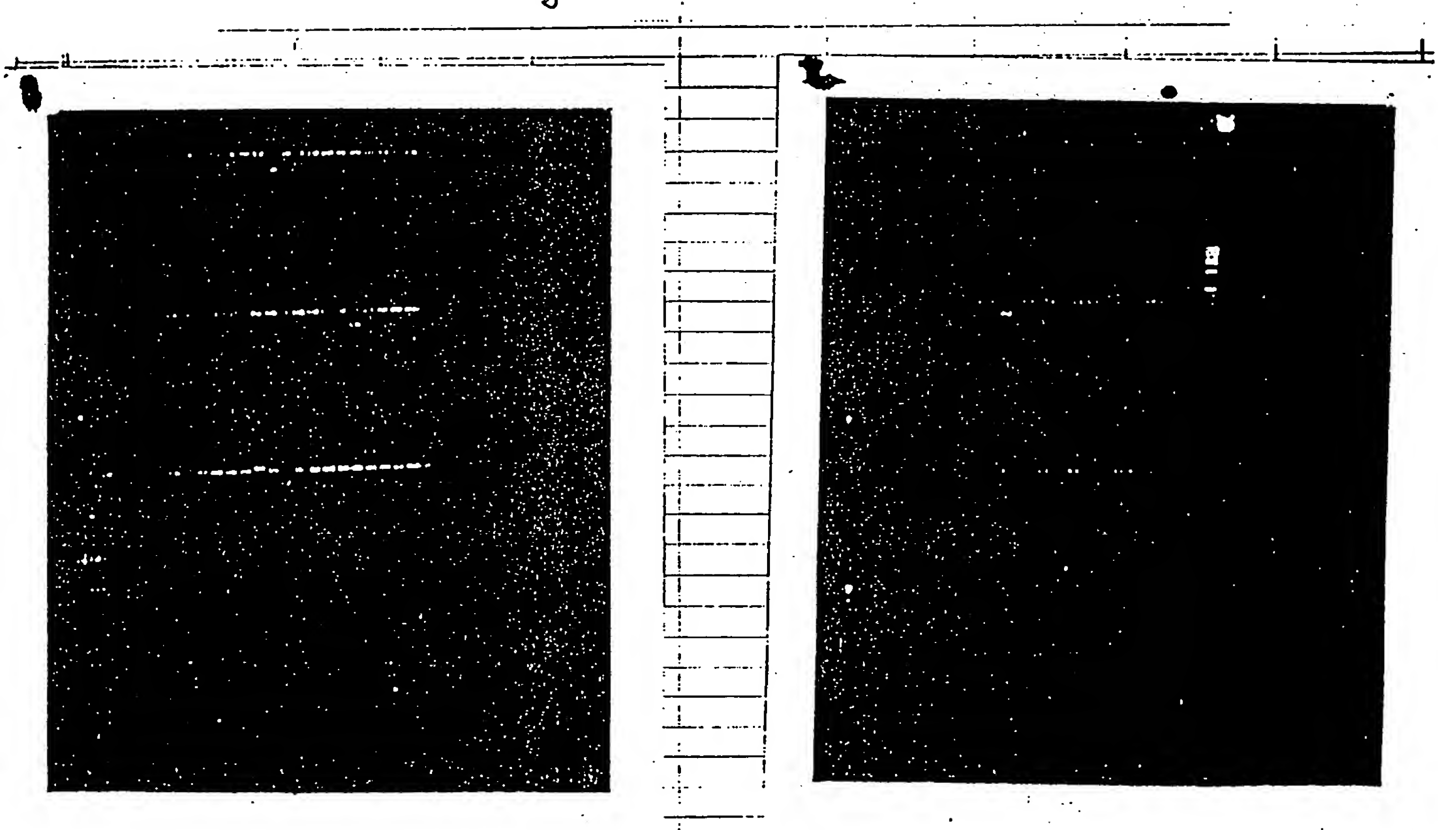


Figure 9

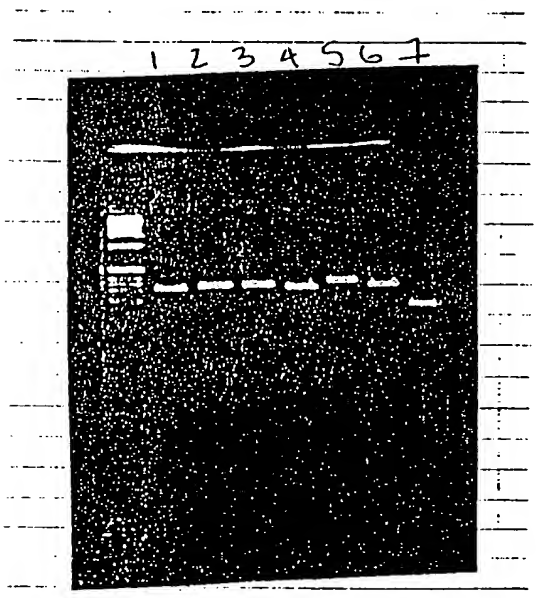


Figure 10

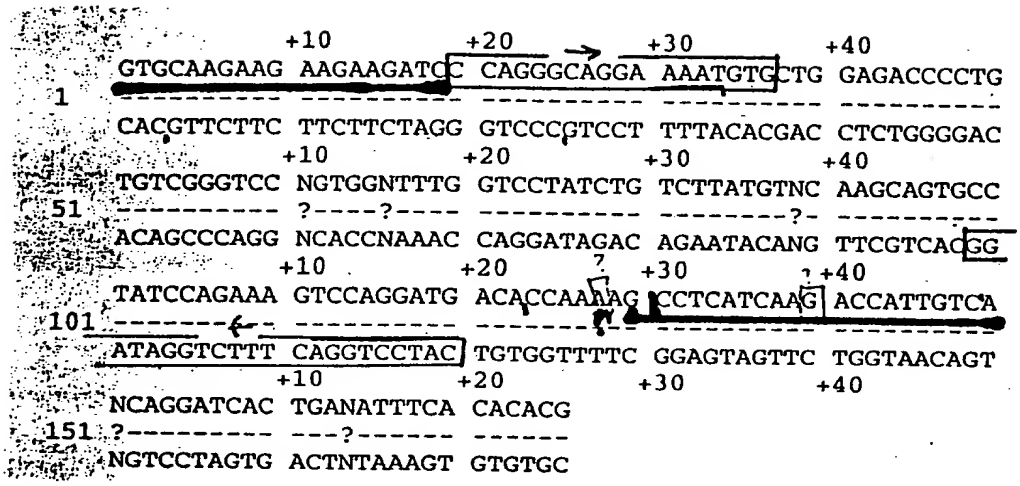


Figure 11A

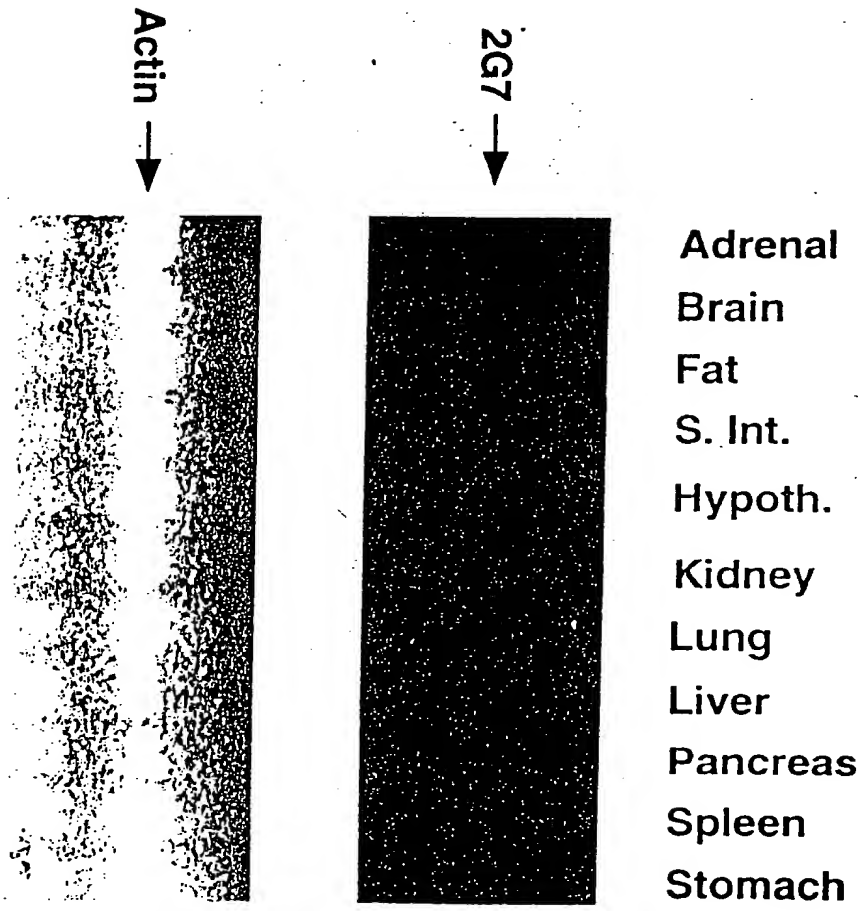


Figure 11B

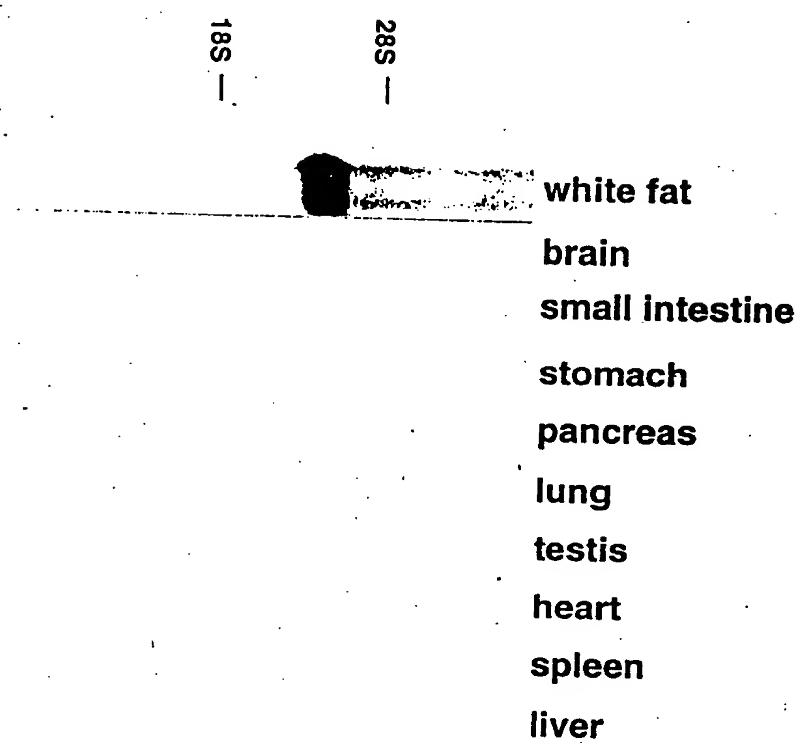


Figure 12A

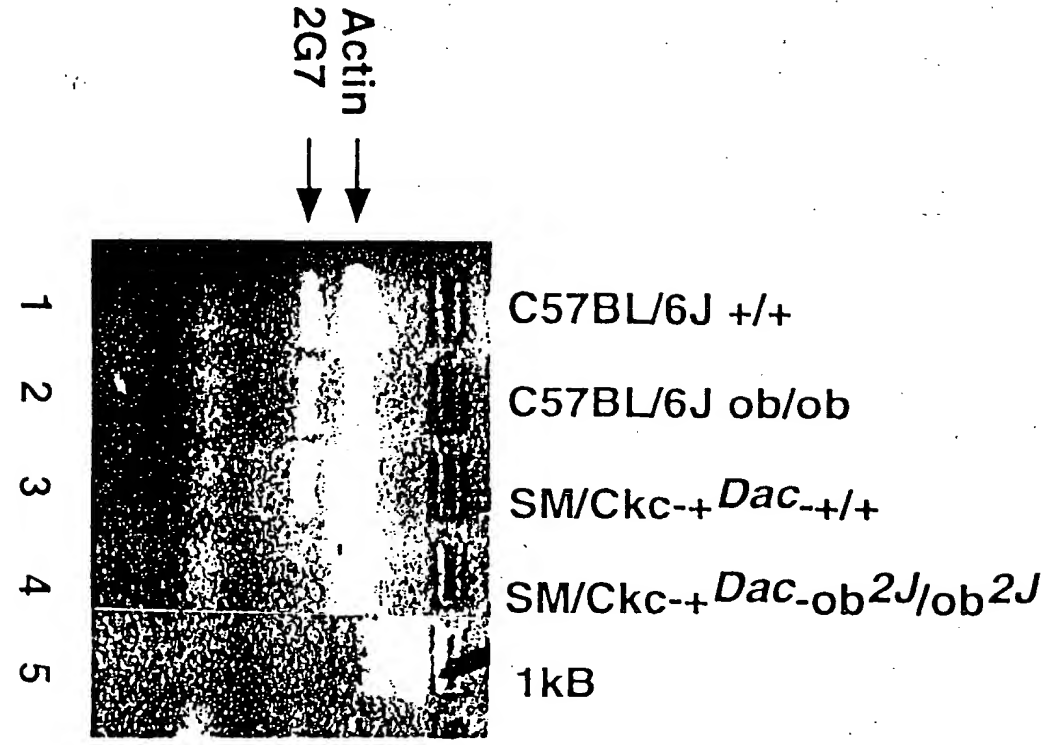


Figure 12 B

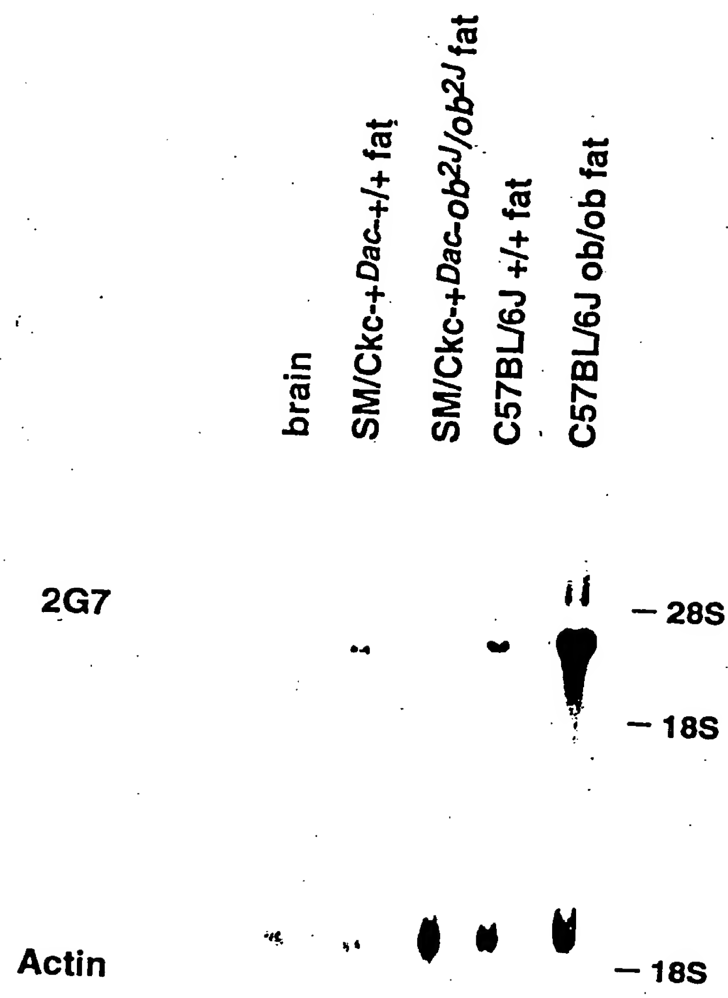

brain
SM/Ckc-+Dac-+/+ fat
SM/Ckc-+Dac-ob2J/ob2J fat
C57BL/6J +/+ fat
C57BL/6J ob/ob fat
2G7
— 28S
— 18S
Actin
— 18S

Figure 13

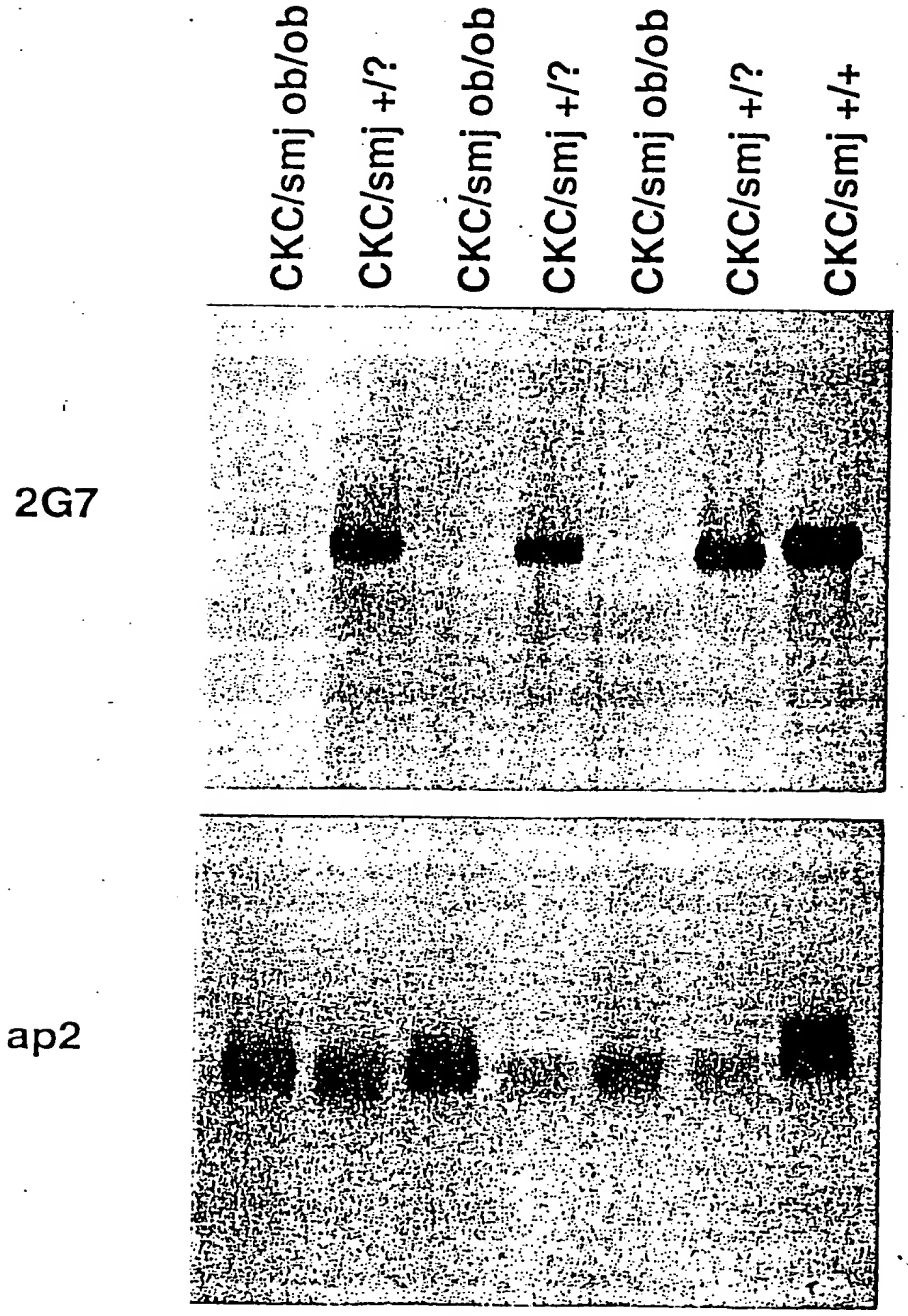


Figure 14

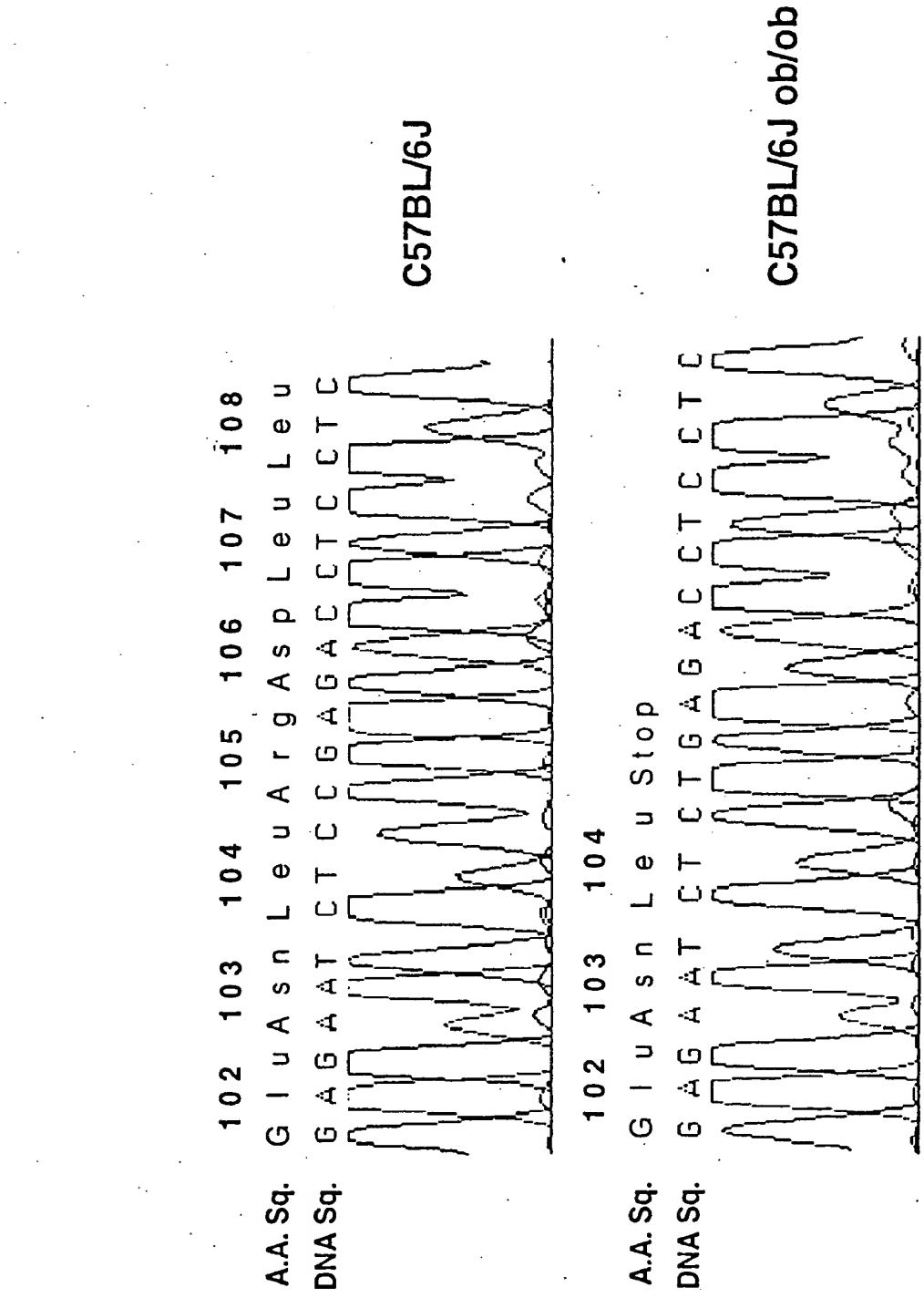

C57BL/6J
A.A. Sq.
DNA Sq.
102 103 104 105 106 107 108
Glu Asn Leu Arg Asp Leu Leu
GAG AAT CT C CG AG AC CT C CT C
C57BL/6J ob/ob
A.A. Sq.
DNA Sq.
102 103 104
Glu Asn Leu Stop
GAG AAT CT CT G AG AC CT C CT C

Figure 15A

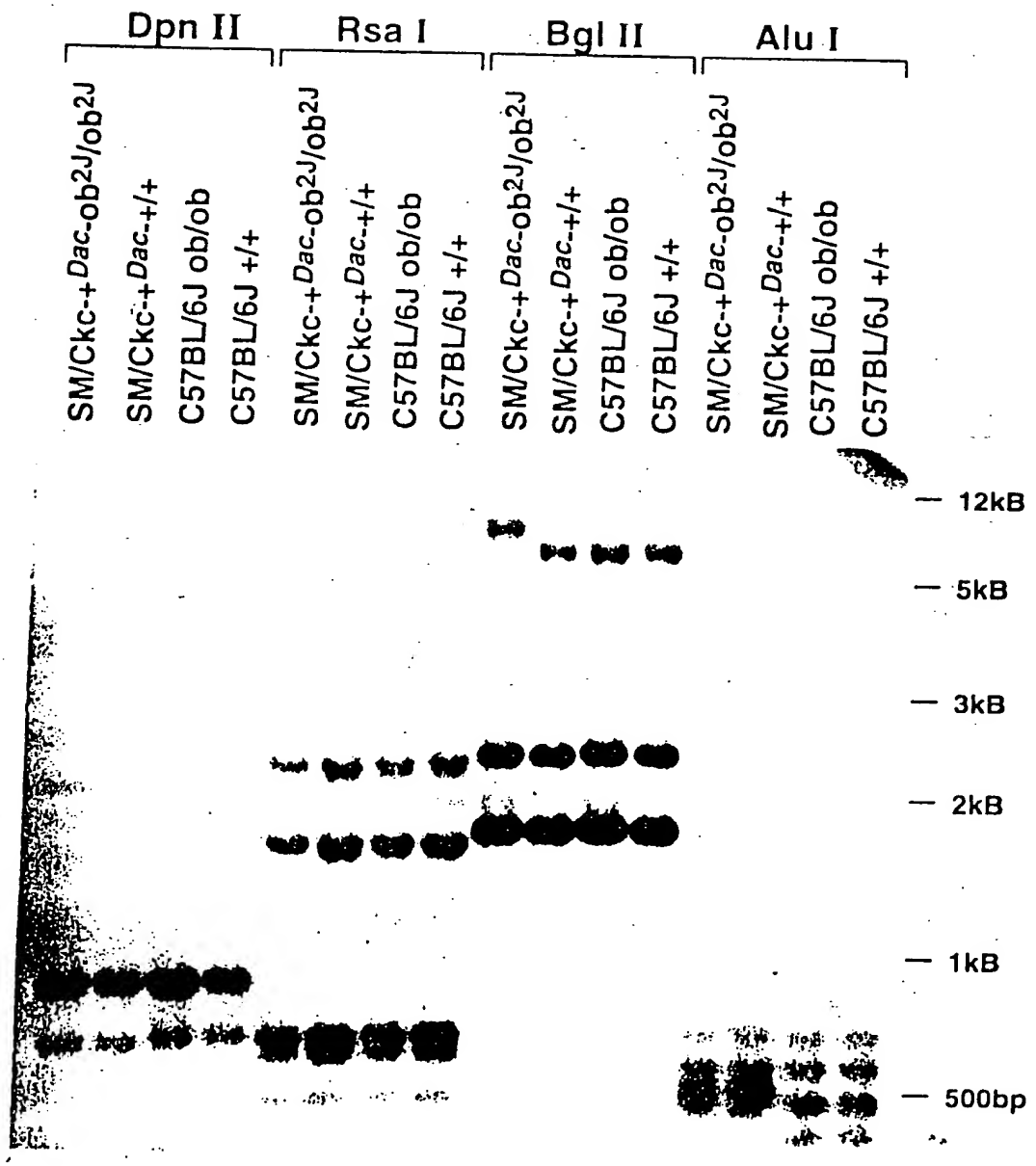


Figure 15B

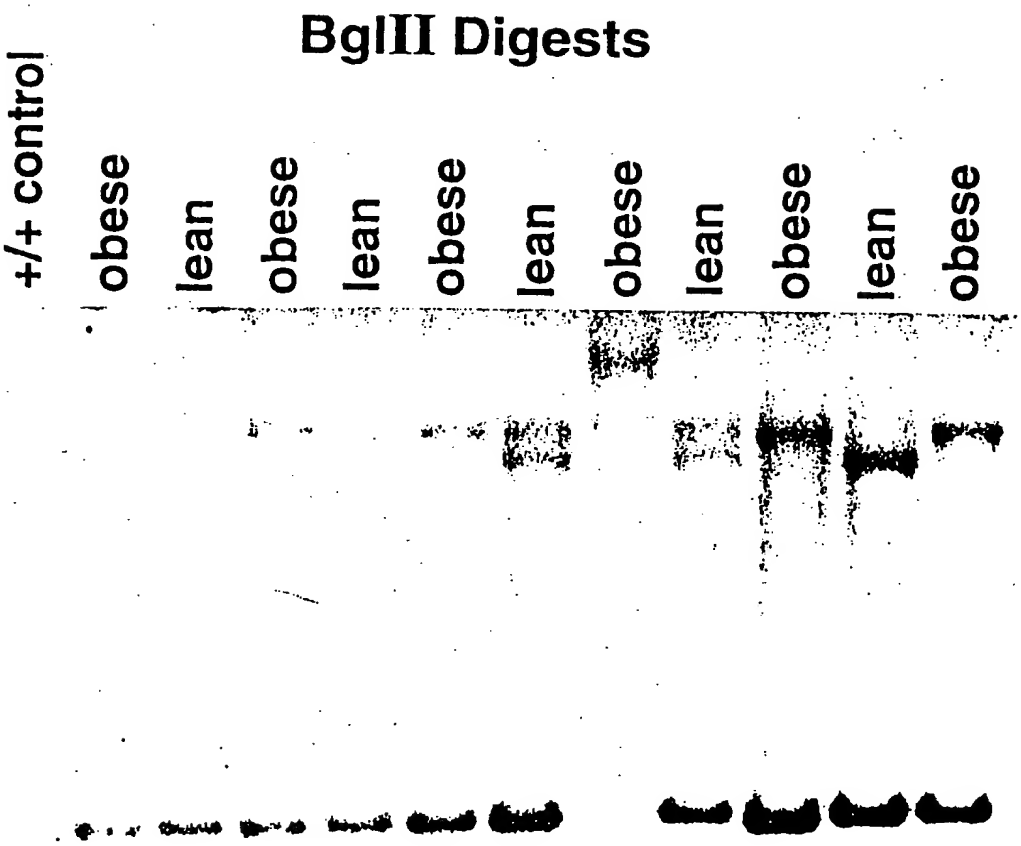


Figure 16

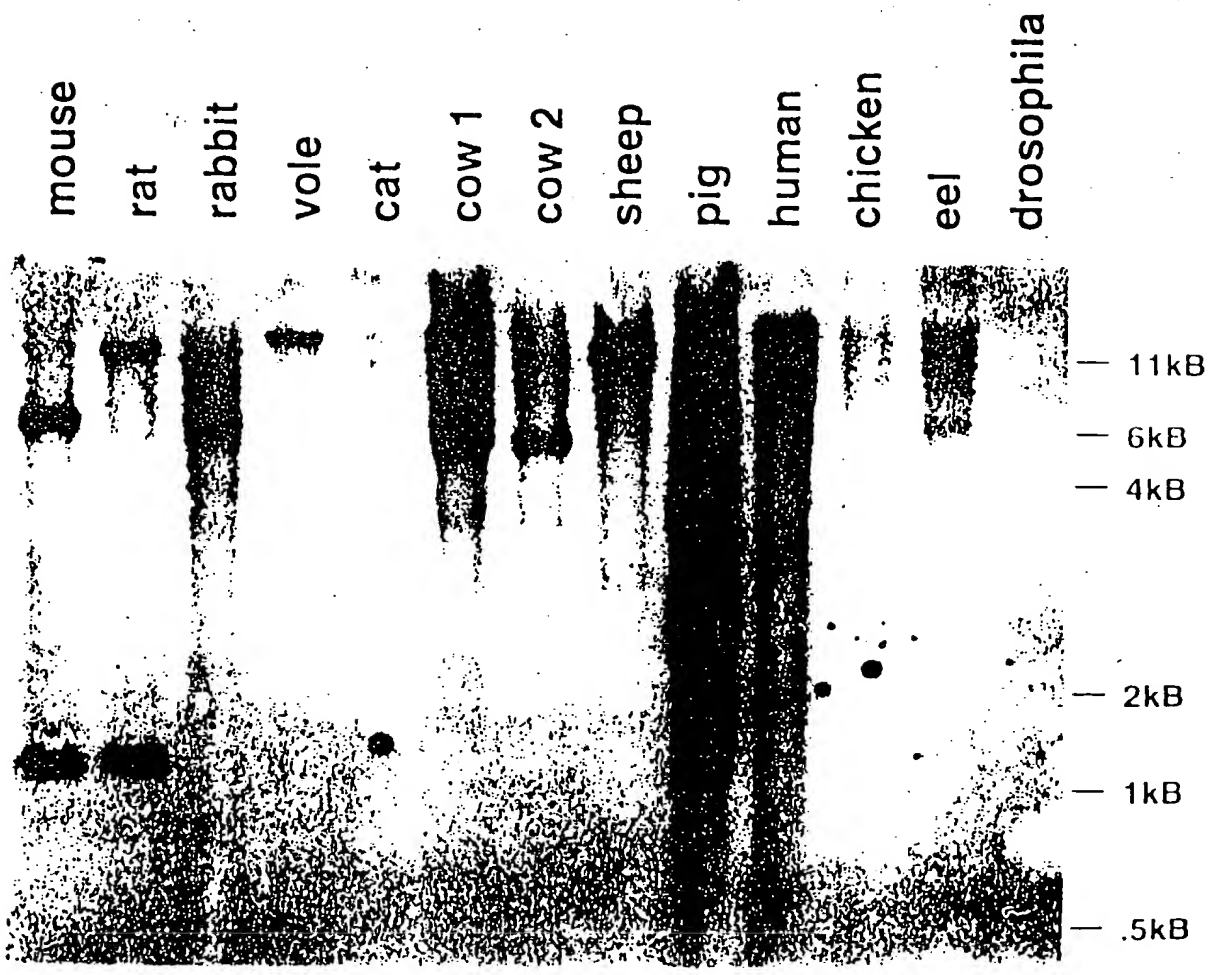


Figure 17

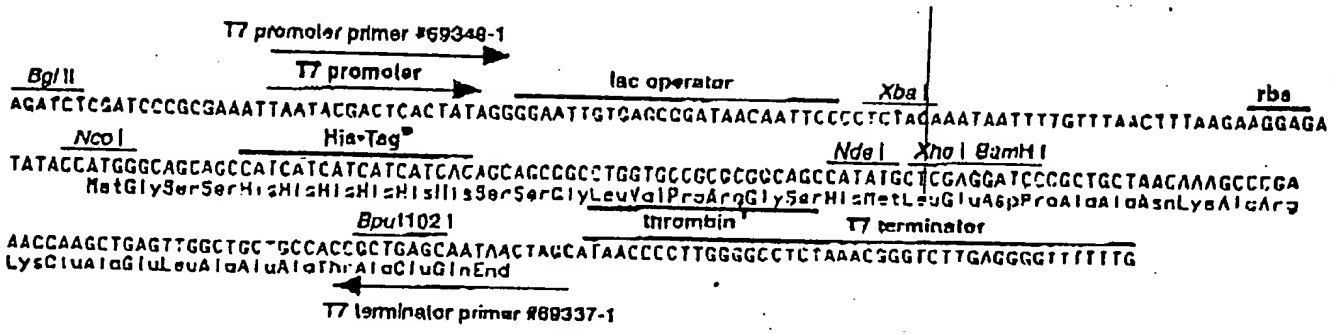


Figure 18A

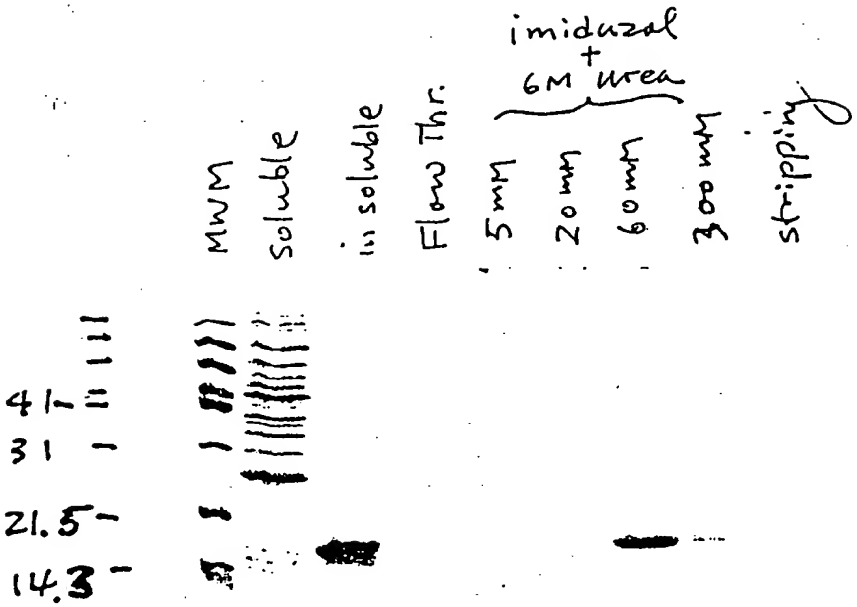
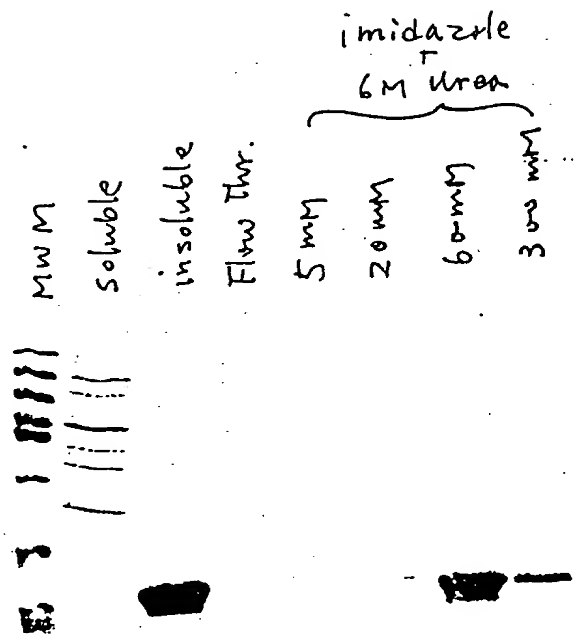


Figure 18B



600-1-081 CIP1 (sheet 23 of 52)

Figure 19A

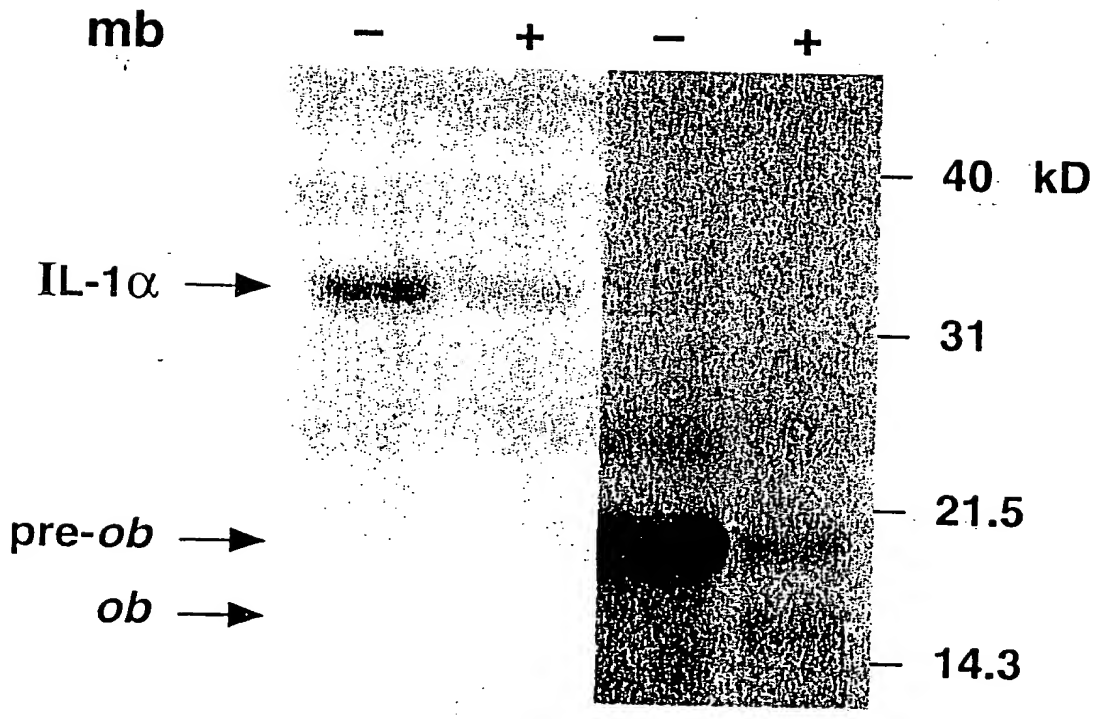


Figure 19B.

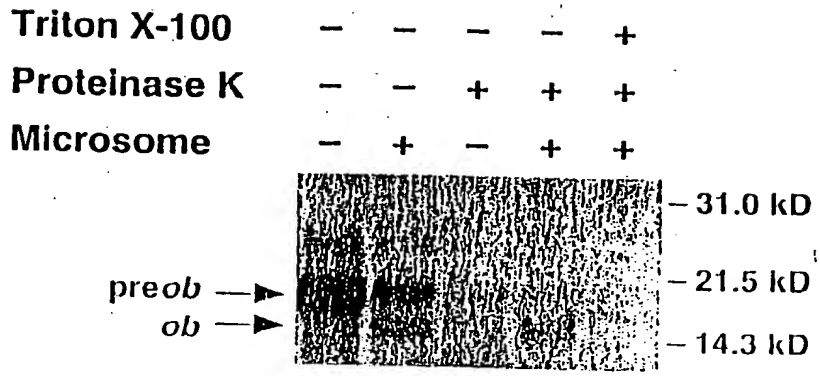


Figure 20A

```
10          20          30          40          50
GGTTGCAAGG CCCAAGAAGC CCATCCTGGG AAGGAAAATG CATTGGGGAA
           HOB1g F                       START
60          70          80          90          100
CCCTGTGCGG ATTCTTGTGG CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT
110         120         130         140         150
GTGCCCATCC AAAAAGTCCA AGATGACACC AAAACCCTCA TCAAGACAAT
                                5' of 1st INTRON
160         170         180         190         200
TGTCACCAGG ATCAATGACA TTTCACACAC GGTAAGGAGA GTATGCGGGG
210         220         230         240         250
ACAAAGTAGA ACTGCAGCCA GCCCAGCACT GGCTCCTAGT GGCACTGGAC
                                             HOB1g R
260         270         280         290         300
CCAGATAGTC CAAGAAACAT TTATTGAACG CCTCCTGAAT GCCAGGCACC
310         320         330         340         350
TACTGCAAGC TGAGAAGGAT TTTGGATAGC ACAGGGCTCC ACTCTTTCTG
360         370         380         390         400
GTTGTTTCTT NTGGCCCCCT CTGCCTGCTG AGATNCCAGG GGTTAGNGGT
410         420         430         440         450
TCTTAATTCC TAAA----GAP OF SEQUENCE (~1.4 kb)--------CT
460         470         480         490         500
GGTTCTTTCA GGAAGAGGCC ATGTAAGAGA AAGGAATTGA CCTAGGGAAA
510         520         530         540         550
ATTGGCCTGG GAAGTGGAGG GAACGGATGG TGTGGGAAAA GCAGGAATCT
560         570         580         590         600
CGGAGACCAG CTTAGAGGCT TGGCAGTCAC CTGGGTGCAG GANACAAGGG
610         620         630         640         650
CCTGAGCCAA AGTGGTGAGG GAGGGTGGAA GGAGACAGCC CAGAGAATGA
660         670         680         690         700
CCCTCCATGC CCACGGGGAA GGCAGAGGGC TCTGAGAGCG ATTCCTCCCA
                      3' of 1st INTRON
710         720         730         740         750
CATGCTGAGC ACTTGTTCTC CCTCTTCCTC CTNCATAGCA GTCAGTCTCC
HOB2g F
760         770         780         790         800
TCCAAACAGA AAGTCACCGG TTTGGACTTC ATTCCTGGGC TCCACCCCAT
810         820         830         840         850
CCTGACCTTA TCCAAGATGG ACCAGACACT GGCAGTCTAC CAACAGATCC
860         870         880         890         900
TCACCAGTAT GCCTTCCAGA AACGTGATCC AAATATCCAA CGACCTGGAG

910         920         930         940         950
AACCTCCGGG ATCTTCTTCA CGTGCTGGCC TTCTCTAAGA GCTGCCACTT
960         970         980         990         1000
GCCCTGGGCC AGTGGCCTGG AGACCTTGGA CAGCCTGGGG GGTGTCCTGG
1010        1020        1030        1040        1050
AAGCTTCAGG CTACTCCACA GAGGTGGTGG CCCTGAGCAG GCTGCAGGGG
1060        1070        1080        1090        1100
TCTCTGCAGG ACATGCTGTG GCAGCTGGAC CTCAGCCCTG GGTGCTGAGG
                                               STOP
1110        1120        1130        1140        1150
CCTTGAAGGT CACTCTTCCT GCAAGGACTA CGTTAAGGGA AGGAACTCTG
1160        1170        1180        1190        1200
GCTTCCAGGT ATCTCCAGGA TTGAAGAGCA TTGCATGGAC ACCCCTTATC
            HOB2g R
1210        1220        1230        1240        1250
CAGGACTCTG TCAATTTCCC TGACTCCTCT AAGCCACTCT TCCAAAGG
```

09583364.081000

Figure 20B

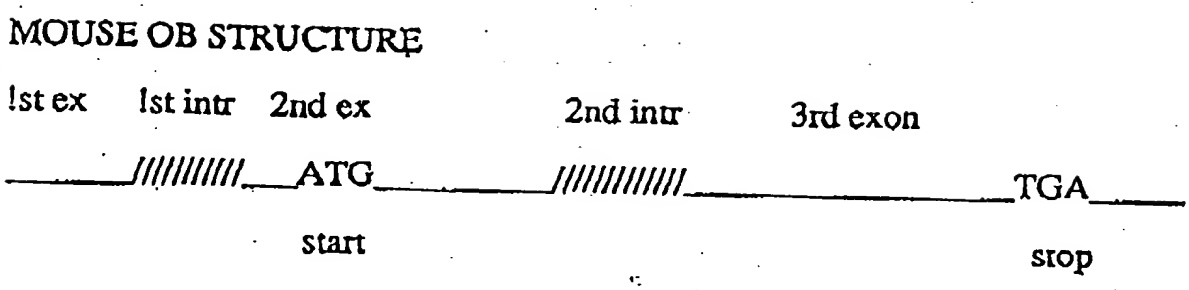


Figure 20c

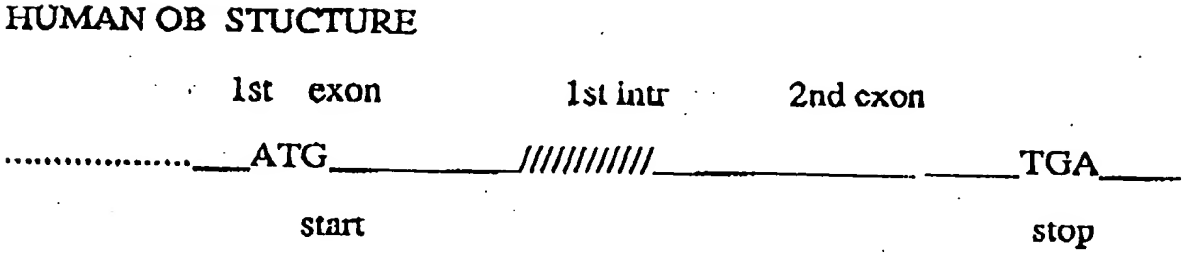


Figure 21A

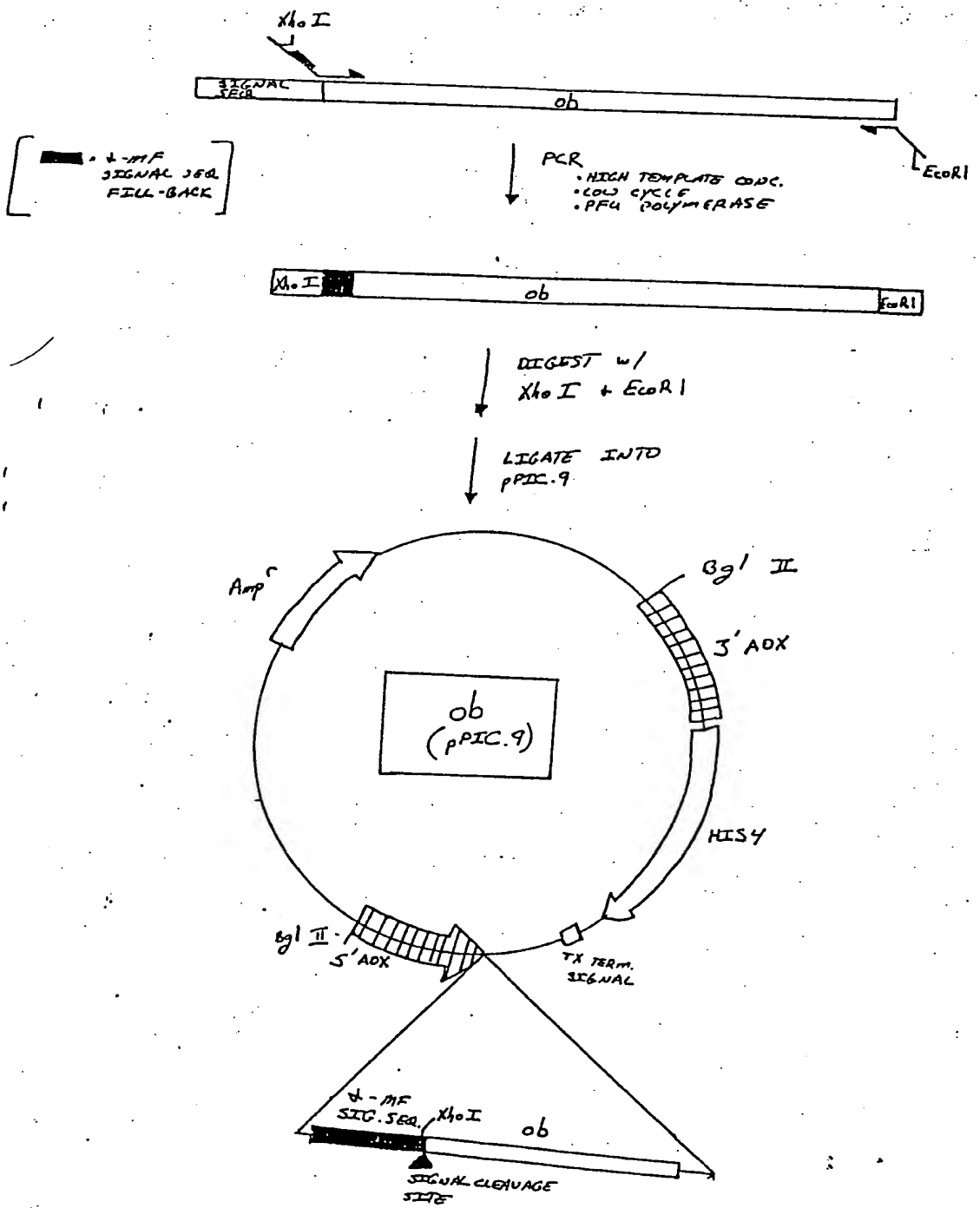


Figure 21 B

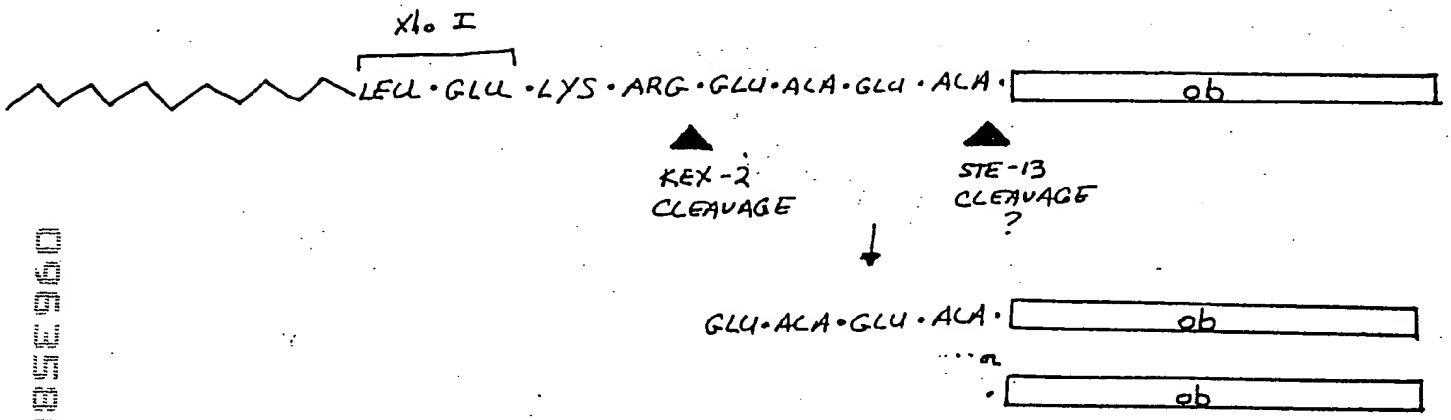


Figure 21 c

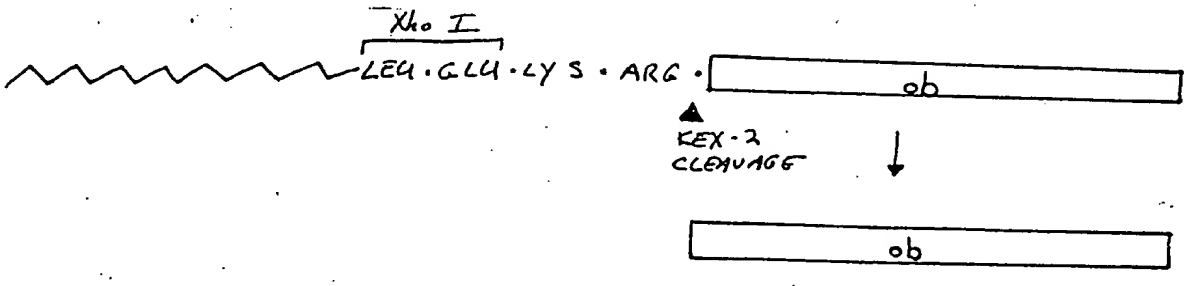


Figure 22A

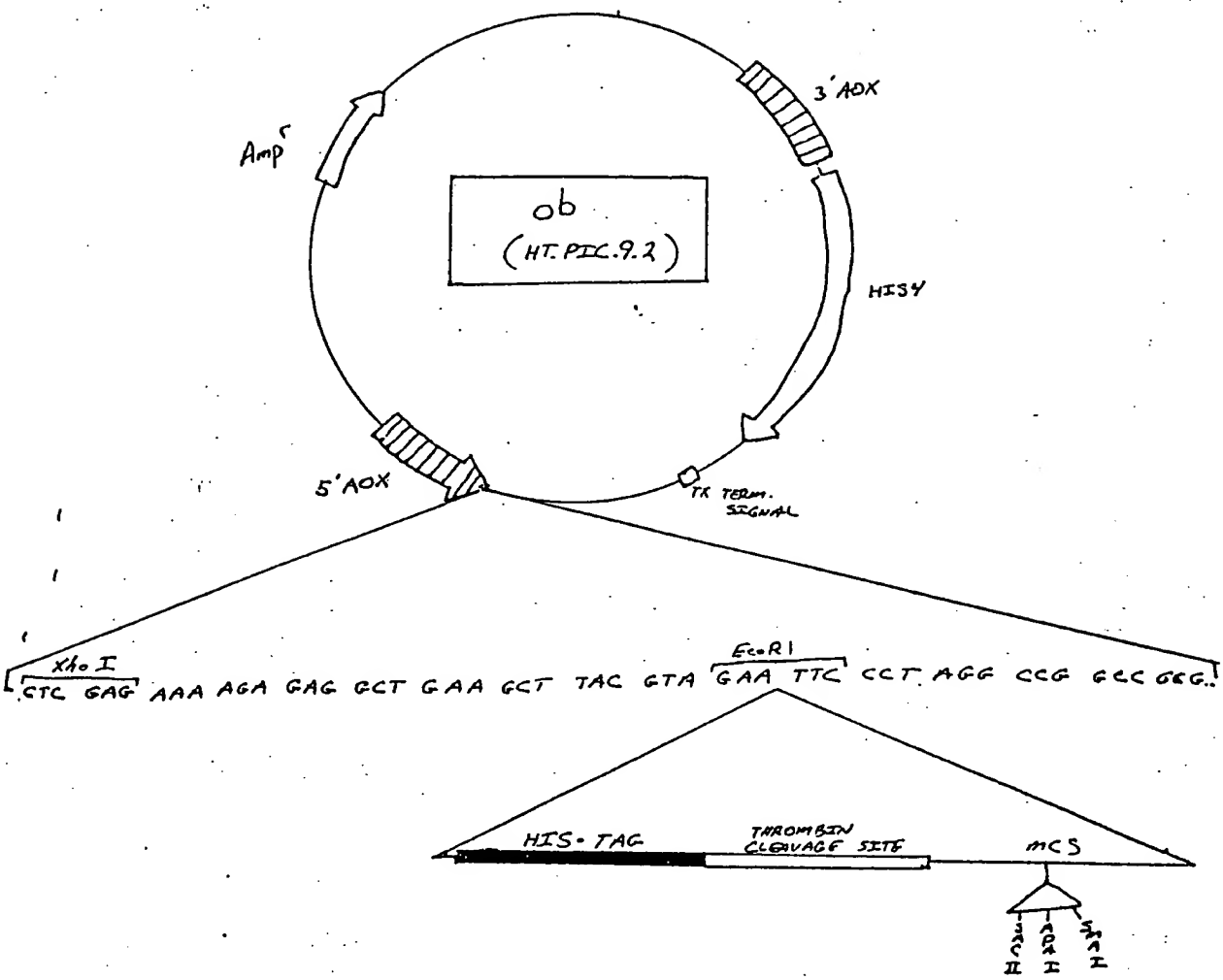

ob
(HT. PIC. 9.2)
Amp^r
3' AOX
HIS4
5' AOX
TX TERM. SIGNAL
Xho I
CTC GAG AAA AGA GAG GCT GAA GCT TAC GTA GAA TTC CCT AGG CCG GCC GCG
EcoR I
HIS · TAG
THROMBIN CLEAVAGE SITE
MCS

Figure 22B

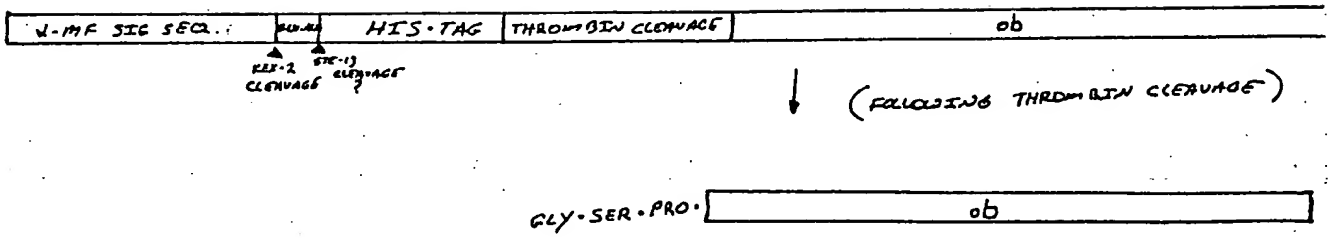

M-MF SIG SEQ..
HIS·TAG
THROMBIN CLEAVAGE
ob
KEX-2 CLEAVAGE
(FOLLOWING THROMBIN CLEAVAGE)
GLY·SER·PRO·
ob

Figure 23A-

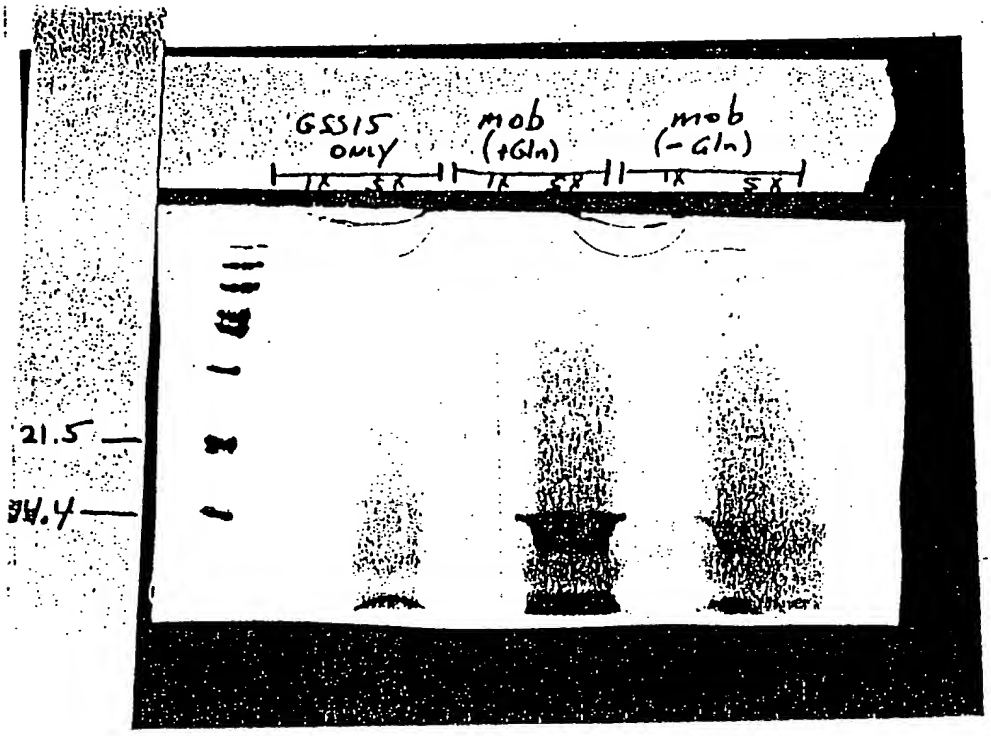


Figure 23B

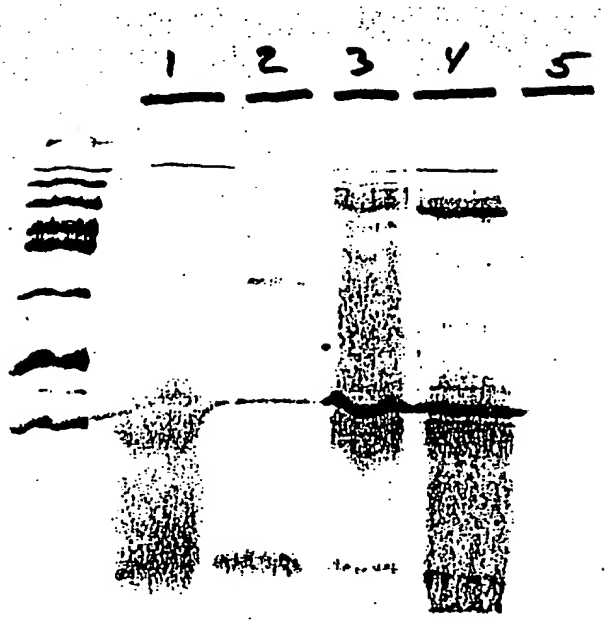


Figure 24 A

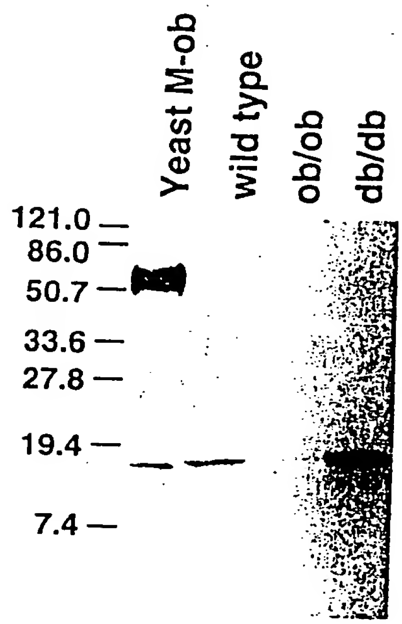


Figure 24B

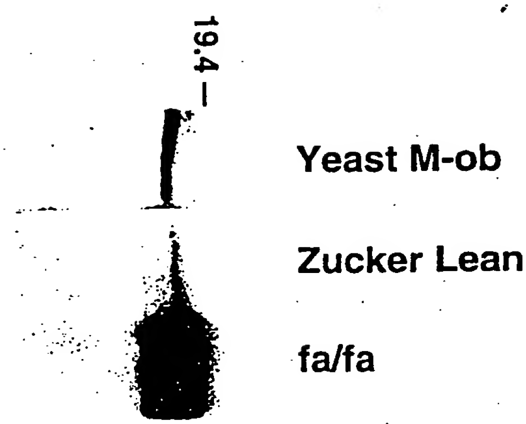


Figure 24C

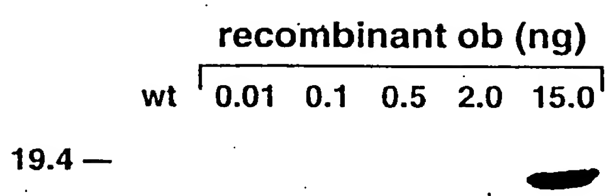


Figure 24 D

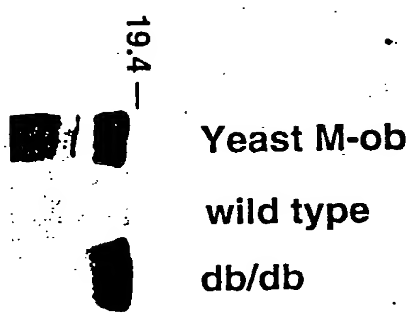


Figure 25A

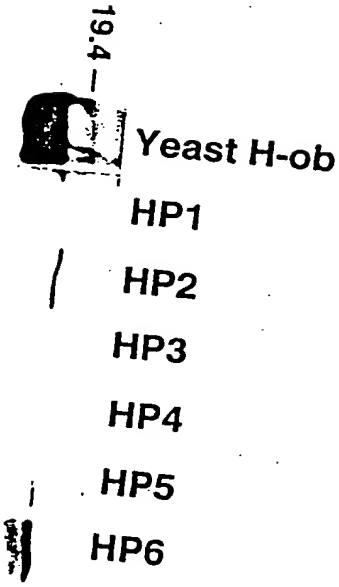


Figure 25 B

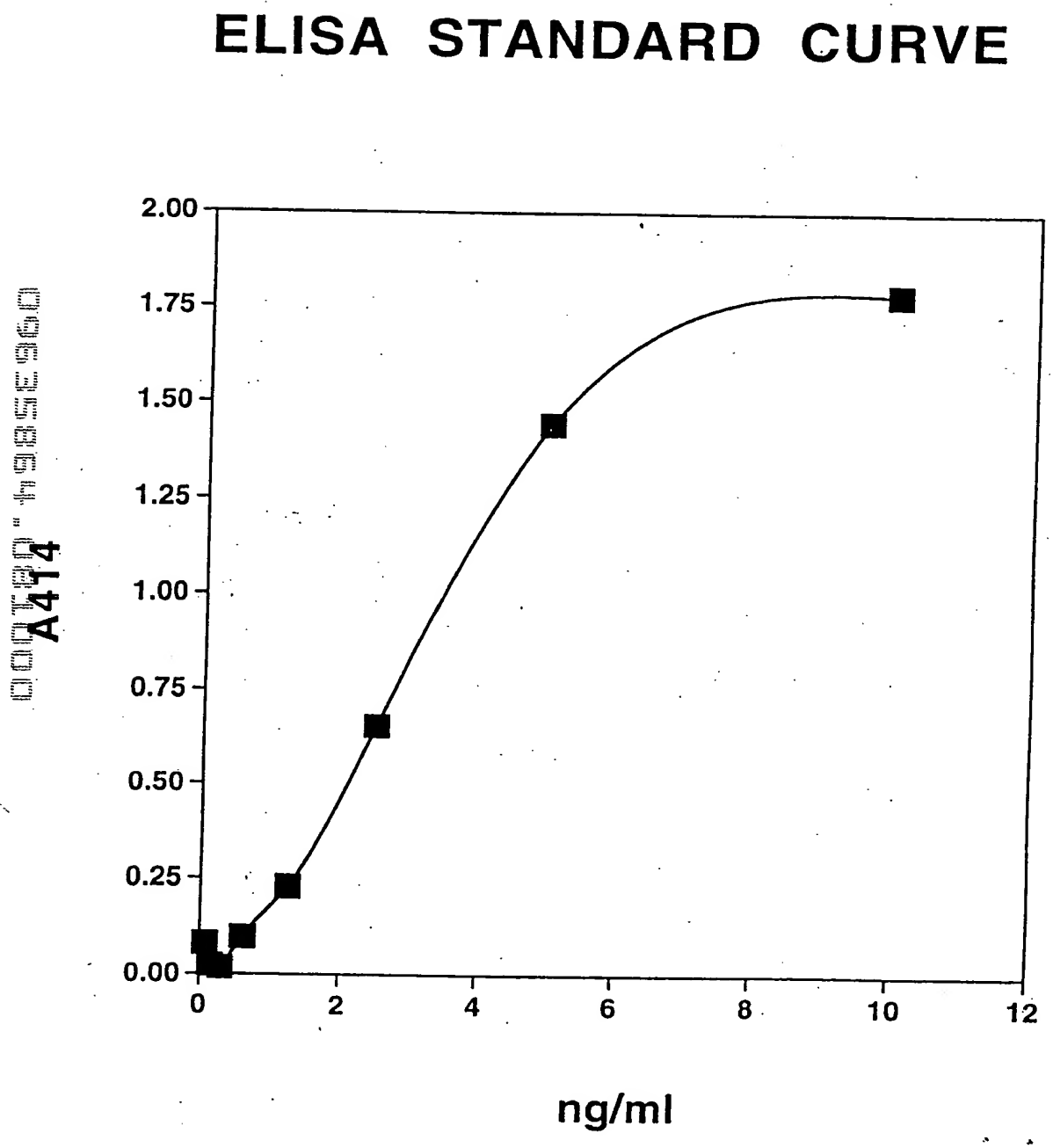
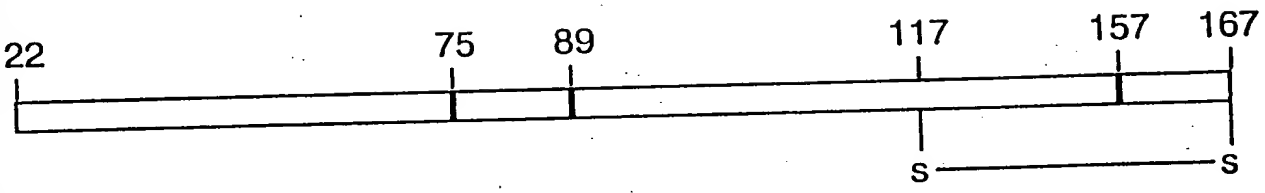

ELISA STANDARD CURVE
A414
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
ng/ml

Figure 26 B

Human ob



Peptide	Mass(Da)	
	Expected	Observed
22-167	16,024	16,024 ± 3
22-75	5936.9	5936.6 ± 1
76-89	1562.7	N.D.
90-167	8434.5	8435.6 ± 1
158-167	1131.9	N.D.

Figure 27

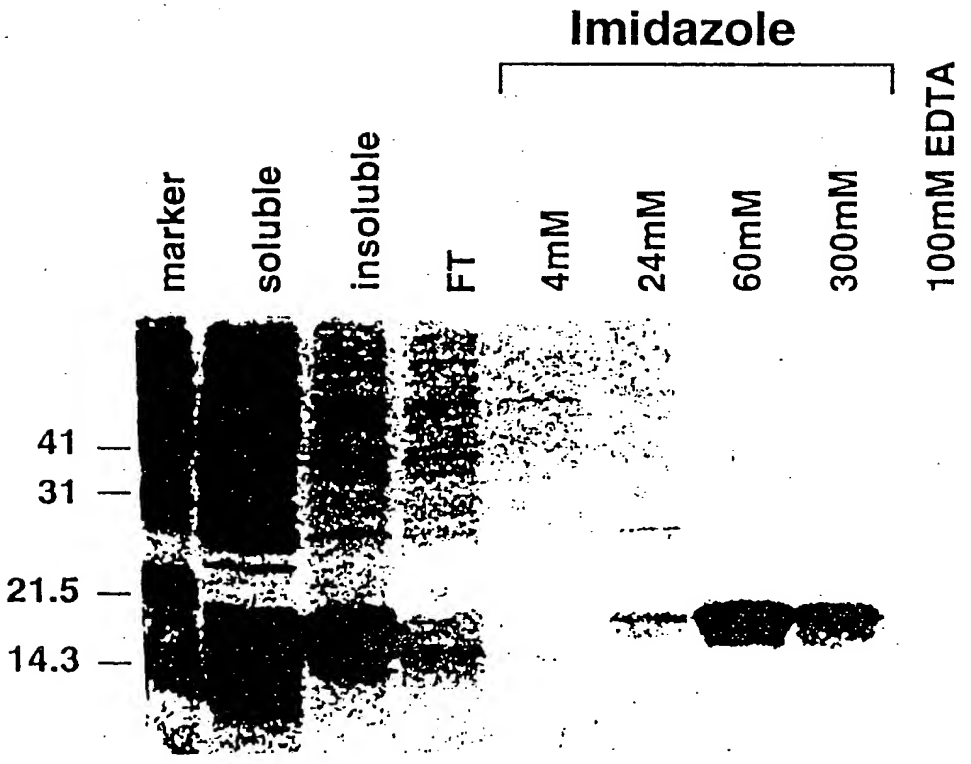


Figure 28 C

Figure 28D

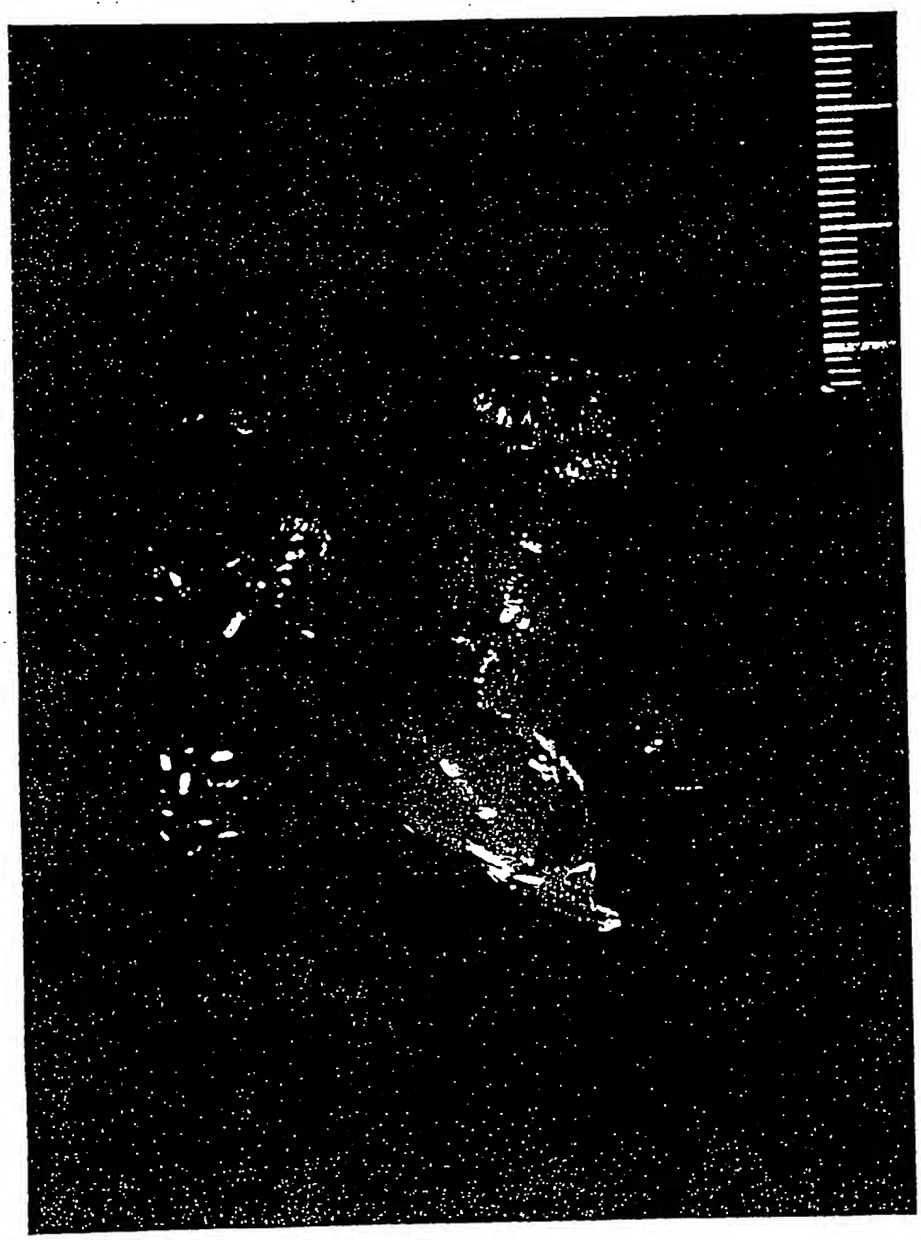


Figure 29

Wt

db/db

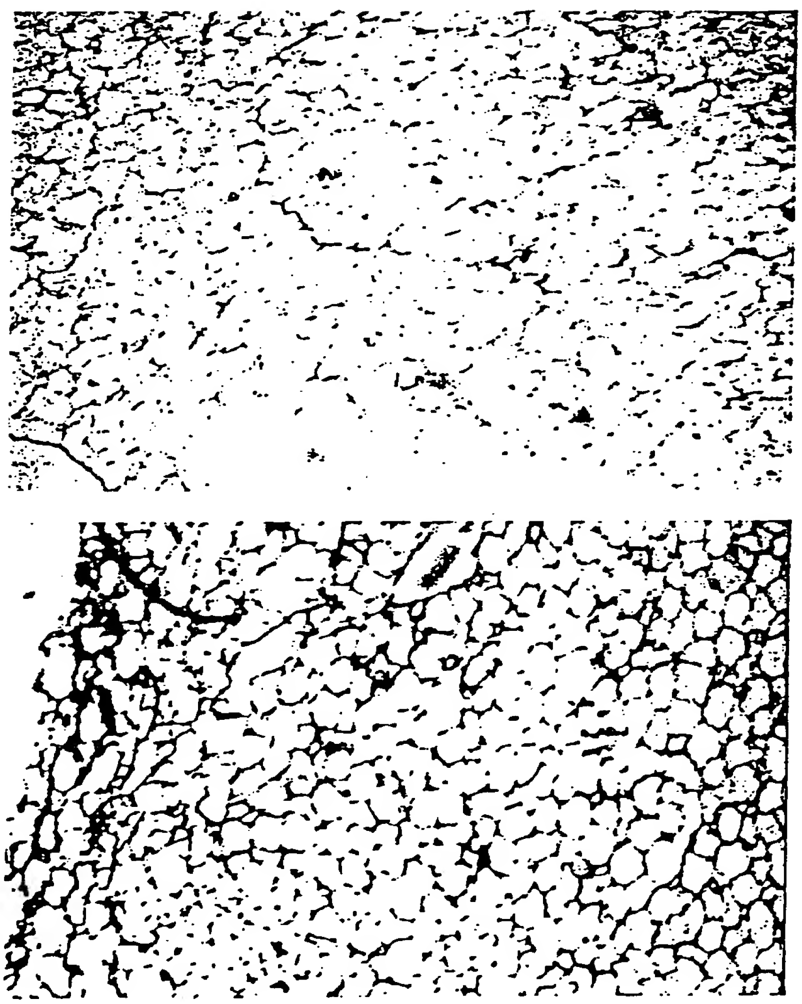


Figure 30

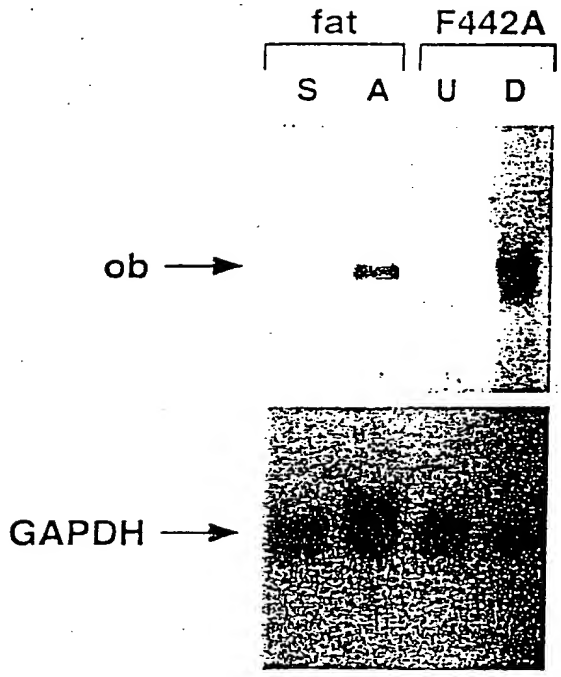


Figure 31 B

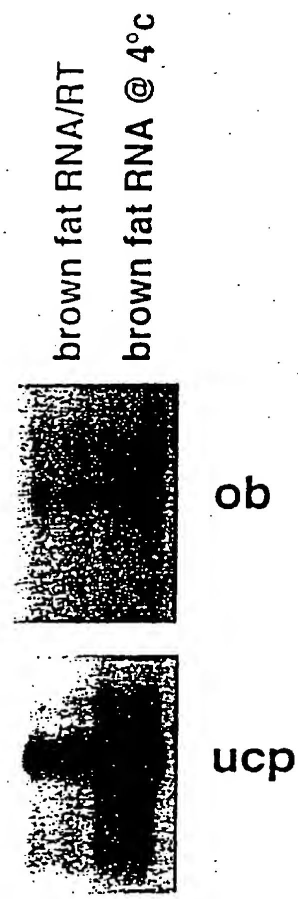


Figure 28B

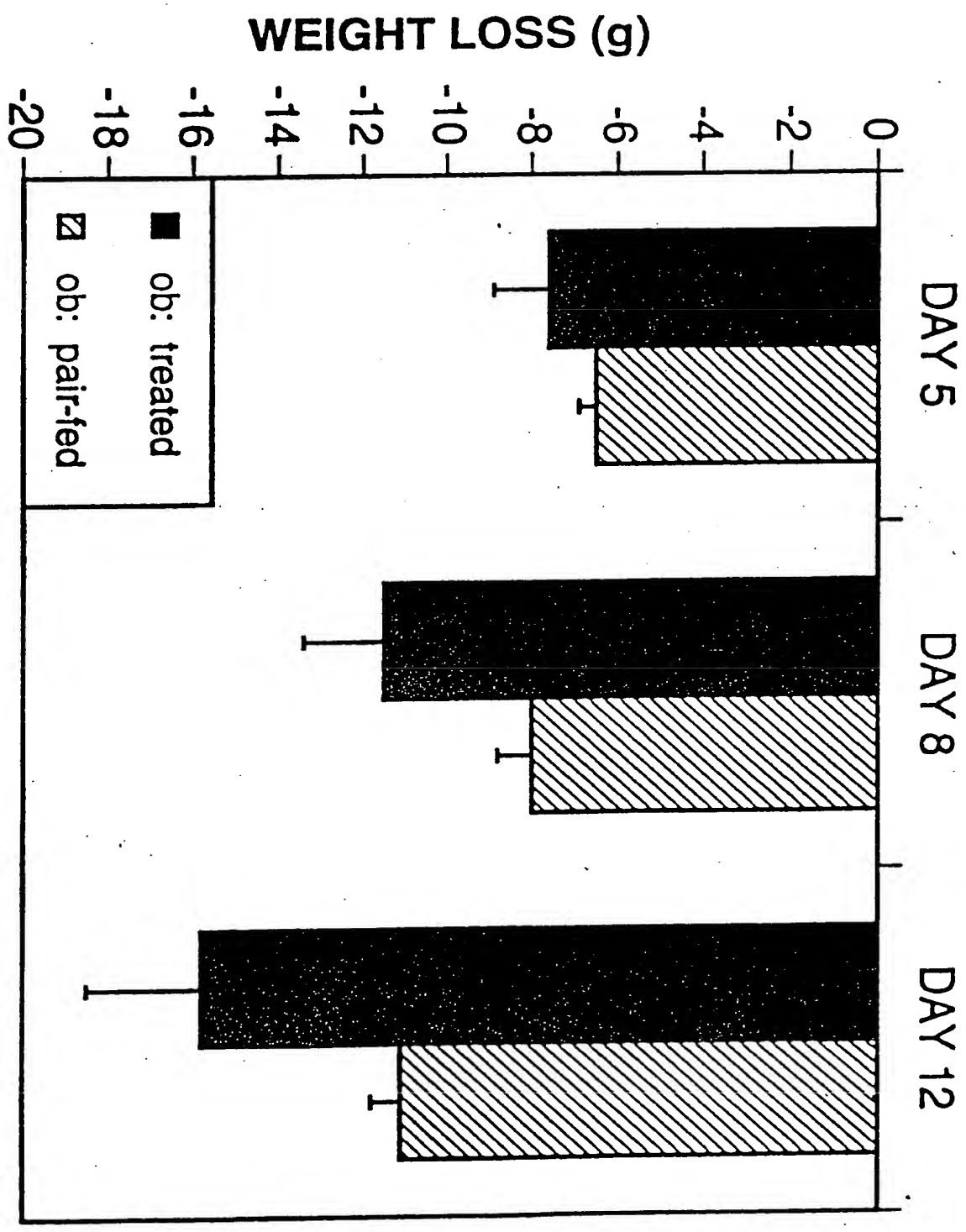


Figure 31 A

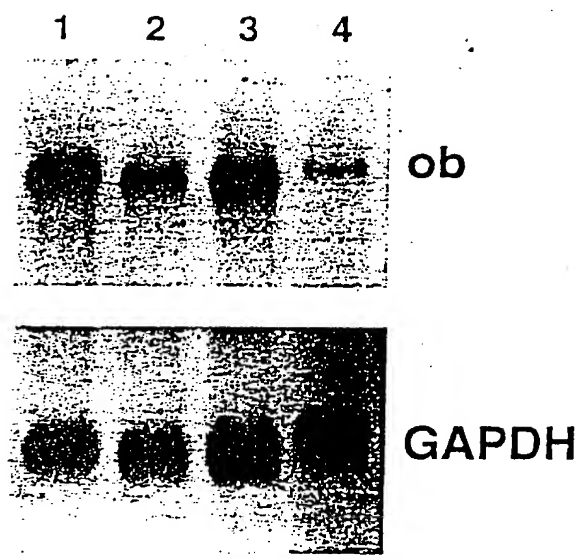
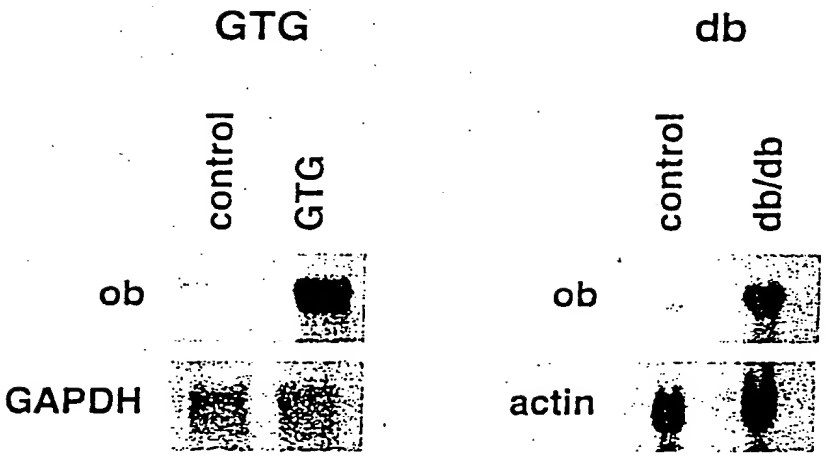


Figure 32



600-(387 LHPI (sheet 31 of 32)

Figure 33

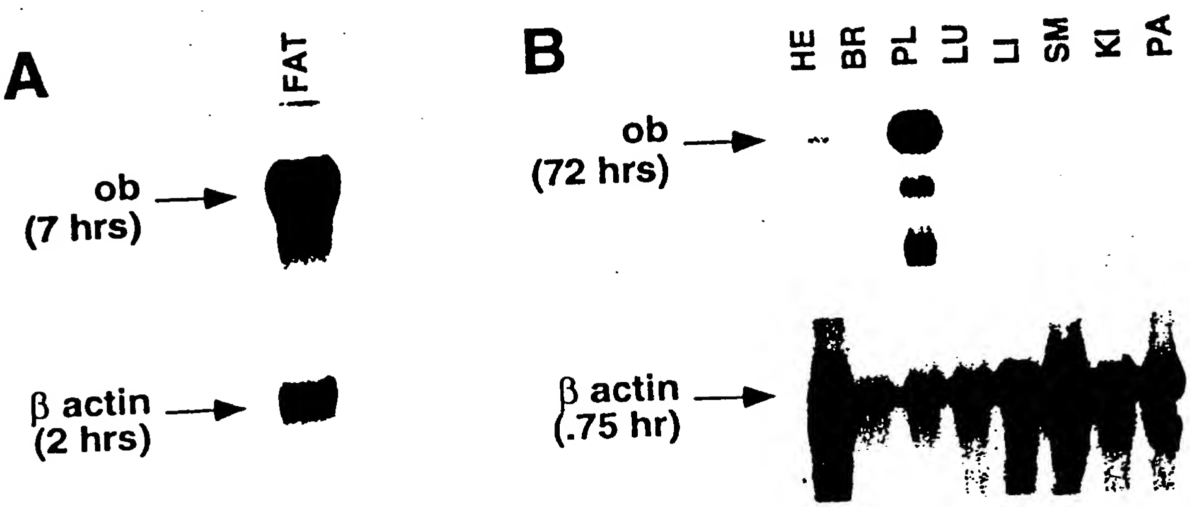


Figure 34